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Molecular systematics of western North American cyprinids (Cypriniformes: Cyprinidae)

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Abstract

The phylogenetic or evolutionary relationships of species of Cypriniformes, as well as their classification, is in an era of flux. For the first time ever, the Order, and constituent Families are being examined for relationships within a phylogenetic context. Relevant findings as to sister-group relationships are largely being inferred from analyses of both mitochondrial and nuclear DNA sequences. Like the vast majority of Cypriniformes, due to an overall lack of any phylogenetic investigation of these fishes since Hennig's transformation of the discipline, changes in hypotheses of relationships and a natural classification of the species should not be of surprise to anyone. Basically, for most taxa no properly supported phylogenetic hypothesis has ever been done; and this includes relationships with reasonable taxon and character sampling of even families and subfamilies. As such, like others, many western North American cyprinid genera have had a controversial taxonomic and systematic history.

Our effort to better understand the evolutionary history of this artificial geographic grouping of species (Western) surveyed taxa and characters broadly. We analyzed 127 taxa (71 species) from 36 genera, including representative taxa from all 22 western genera hypothesized to form the Western Clade *sensu* Coburn and Cavender (1992). Our evaluation also included additional sampling from a heterogeneous array of species from the western genera *Algansea*, *Gila*, *Lepidomeda*, *Ptychocheilus* and *Siphateles*. Resulting phylogenetic inferences, based on one mitochondrial and three nuclear genes (mtDNA: *cytb*; nDNA: *Rag1*, *Rhod*, *S7*), consistently resolved a well-supported Western Clade, but one inclusive of *Chrosomus erythrogaster*. This taxon, always formed the sister group to the extant species of *Gila* plus 10 other western genera. Our Western Clade is qualitatively different from that of prior studies and does not include the genera *Agosia*, *Algansea*, *Iotichthys*, *Lepidomeda*, *Meda*, *Mylocheilus*, *Plagopterus*, *Pogonichthys*, *Rhinichthys*, *Tiaroga* or *Yuriria*. All of these taxa were, however, included in Coburn and Cavender's (1992) Western Clade. Our broader-scale survey and increased character sampling were always resolved these latter taxa within one of two different major clades: the OPM Clade (*sensu* Mayden 1989) and the Creek Chub—Plagopterin Clade (*sensu* Simons *et al.* 2003). Our hypothesized Western Clade places *Orthodon* sister to a Western Chub-Pikeminnow Clade also inclusive of *Acrocheilus*, *Eremichthys*, *Gila*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, *Ptychocheilus*, *Relictus* and *Siphateles*. The latter taxa have traditionally been recognized at the generic level, simply on the basis of their morphological distinctiveness and not on the basis of a phylogenetic evaluation of relationships. Composition of our Western Chub-Pikeminnow Clade also reveals genetic divergences between species of some genera (*Gila*, *Ptychocheilus*, *Siphateles*) comparable to genetic divergences documented between genera within the Western Clade. Relationships for these 10 genera also highlight taxonomic inconsistencies relative to recent phylogenetic analysis and, in some cases, are in need of focused attention using morphology or additional molecular data to test relationships that will eventually establish a stable classification. Some of these genera are clearly unnatural relative to other genera and their classification or ranking is an obligatory change in modern science of phylogenetics.

Key words: Western Clade, Phoxinins, sequence analyses, *Gila* lineage, controversial taxonomy

Introduction

The evolutionary history of native western North American freshwater fishes is inextricably linked to a series of disruptive geologic and climatic events (Smith 1981; Smith *et al.* 2002; Minckley *et al.* 1986). Together these events have been historically hypothesized to have lead to the evolution of a remarkably distinctive cyprinid fauna, evidenced by a high percentage of endemic species and genera. Curiously, unlike other faunas of the world, roughly 50% of the genera in the west are monotypic (Miller 1959), an observation worthy of further investigation given the time of his writing. These western North American taxa, like many groups at that time, presented researchers with several taxonomic questions/problems. Even today, following the emergence of phylogenetic systematic methods, taxa lacking any systematic evaluation can be equally perplexing; although the same is true of groups that have received limited attention in terms of taxon and character sampling. Western taxa are highly variable in appearance, biology, and distribution, ranging from small herbivorous species (*Eremichthys*, 65 mm SL) to large piscivorous species (*Ptychocheilus*, 1.8 m SL). Many of the species have restricted habitats and ranges and are considered vulnerable, imperiled or prone to extinction (U.S. Fish and Wildlife Service 2010; SEDESOL 1994). This includes several members of the genus *Gila* as well as most of the rare monotypic genera (i.e. *Eremichthys*, *Moapa*, *Plagopterus*, *Relictus*).

At the time of this writing nearly five decades have passed since phylogenetic methods arrived in the New World (Mayden 1992; Burr & Mayden 1992) and was being practiced by some ichthyologists here. It was not until roughly the late 1980's–1990's that this method was used at a relatively large-scale to evaluate possible homologous characters and their analysis for North American cyprinids (Mayden 1989; Coburn and Cavender (1992). Up to that time, and to some degree since, it was as Hubbs and Miller (1977) commented when trying to find the natural placement of species of *Dionda*, that much of the classification of North American cyprinids was in a state of chaos. Only by avoiding any new hypotheses of relationships proposed by researchers using phylogenetic methods, either implicitly or explicitly, did the classification of these fishes, and others on the continent, remain “stable.”

With the emergence of initial studies by Mayden (1989) and Coburn and Cavender (1992), and many following to test these hypotheses or provide various levels of species relationships within groups, many changes in the classification developed. For a variety of possible reasons related to homology assessment, types of analyses, assumptions related to analyses, or unrelated preferences in deriving a classification, there have been considerable differences in some areas of the phylogeny of North American cyprinids. However, equally important, there have also been many instances of additional studies corroborating one or more hypotheses put forth in previous studies.

In this study, we provide the most comprehensive molecular phylogenetic study to date designed to test previous hypotheses as to the relationships of the Western Clade and to test its monophyly. To avoid potential problems associated with taxon and character sampling (*sensu* Hillis 1998) we have surveyed a broader spectrum of taxa and examined both mitochondrial and nuclear gene sequences in our approach to these evolutionary inferences and specific tests. As such, we include not only all 22 genera of the Western Clade as proposed by Coburn and Cavender (1992) but includes several other North American cyprinids to test various hypotheses of relationships of taxa across the continent. Our ultimate goal is to reconstruct the phylogenetic relationships among all currently recognized western North American cyprinid genera (natural or not) through observed variation in one mitochondrial and three nuclear DNA genes analyzed using diverse phylogenetic methods. The variable rates of evolution in these four genes has been demonstrated by several authors to make them highly informative in analyses done in large clades with deep and shallow nodes and across a broad range of evolutionary divergences (Quattro *et al.* 2001). This study also extends phylogenetic hypotheses beyond previously published analyses of western cyprinids that potentially suffer from incomplete taxon and character sampling through the addition of multiple species within genera (i.e. *Algansea*, *Gila*, *Lepidomeda*, *Ptychocheilus* and *Siphateles*), the inclusion of 13 nonwestern North American phoxinin genera, closely related Far Eastern cyprinid taxa, and a greater character-based sampling of both mitochondrial and nuclear genes.

Brief Review of Taxonomic and Systematic History

The western cyprinid fauna was historically hypothesized to be related to both Asian (Howes 1984; Miller 1959, 1965) and eastern North American members of the family (Bailey 1956; Uyeno 1960). These studies, however,

lacked either a means of phylogeny reconstruction or included small samples of taxa. Some studies have also rejected the hypotheses that the entire western cyprinid fauna is monophyletic (Coburn & Cavender 1992; Simons *et al.* 2003). The geographic division of the western North American cyprinids, expected with hypotheses of monophyly of all genera west of the continental divide, has also been clearly challenged by well-corroborated hypotheses of sister relationships between some western and eastern genera, including *Richardsonius* and *Clinostomus* (Coburn & Cavender 1992; Simons *et al.* 2003), *Yuriria* and species of south and central Mexican *Notropis* (Schönhuth & Doadrio 2003), and unpublished phylogenies recovering similar relationships for widespread genera (e.g., *Rhinichthys*) inhabiting both eastern and western drainages of North America.

The taxonomy of western cyprinids has had a troublesome history. This history of an unstable classification, even in the face of evidence falsifying earlier hypotheses of divergence-based classification/relationships, have simply been followed, for a variety of reasons, the foremost being an argument of stability in nomenclature. Alternatively, different genera were originally described for species but were subsumed by workers into another same genus without any phylogenetic or evolutionary evidence (Miller 1945a, b, 1959, 1965; Bailey 1956; Miller and Hubbs 1960; Uyeno 1960; Avise & Ayala 1976). Some of these taxonomic anomalies continue today even in the face of contradictory evidence (Mayden 1989; Coburn and Cavender 1992; Simons *et al.* 2003; Schönhuth *et al.* 2008; Bufalino and Mayden 2010a, b, c). The expected evolution of a classification for a group is exemplified by studies of the evolution of genus *Dionda* (as recognized prior to 2008). An appropriate progression of changes in nomenclature, with changes in our understanding of phylogenetic relationships of the group, comes from the transitional studies by Hubbs and Miller (1977) searching to appropriately allocate several new species described from Mexico, to Mayden *et al.*'s (1992) phylogenetic hypothesis of these species, and, finally, to the latest in-depth analyses by Schönhuth *et al.* (2008) of not only *Dionda* but many other North American genera and species. In the first and second studies, either the research was not phylogenetic or taxon and character sampling was really inadequate to properly test relationships, respectively. The latter study rectified these faults through large-scale sampling and multiple sequence data; the end result was that some “*Dionda*” were split out into the new genus *Tampichthys*, containing species that are more closely related to *Codoma* and *Cyprinella* than other “*Dionda*.” The name *Dionda* was retained for species of the *D. episcopa* complex, where the type species resides, and the new classification is insightful as to why a seemingly complex “crevice spawning behavior” thought to be a synapomorphy of the genus *Cyprinella* was observed in *Tampichthys dichromus*.

With the increased attention to fishes in North America some concern has been raised as to varied, contradictory, or seemingly contradictory hypotheses of relationships. This is the natural evolution of hypothetico-deductive model to the scientific method and change is inherent, especially since the fauna has either had no investigations using the phylogenetic method or where done the changes may have been suppressed. However, it should also be noted that appearances of inconsistency may sometime be deceiving and requires more careful scrutiny by the reader. Some unresolved relationships in one analysis, but more fully resolved in another analysis, may actually be consistent with one another, and not inconsistent, as it is just that the former hypothesis contains less information regarding some evolutionary inferences.

In these cases, like that also occurring and underlying any of the world's faunas and floras, differing evolutionary hypotheses regarding members of the “western fauna” have emerged in different studies simply from using different characters, different character/homology interpretations, and/or different types of data analyses (see nonphylogenetic and phylogenetic studies of Miller 1945a, b, 1959, 1965; Bailey 1956; Miller & Hubbs 1960; Bailey 1956; Uyeno 1960; Avise & Ayala 1976; Mayden 1989, Mayden *et al.* 1991; Coburn & Cavender 1992; Simons & Mayden 1997, 1998, 1999; Simons *et al.* 2003; Smith *et al.* 2002; Bufalino & Mayden 2010a, b, c; Houston *et al.* 2010a).

In general, phylogenetic studies have hypothesized that all North American phoxinins (with the exception of *Notemigonus*) fall out into three major clades, but the composition and names of these three clades depend on the individual study (Mayden 1989; Coburn & Cavender 1992; Simons & Mayden 1997, 1998, 1999; Simons *et al.* 2003; Bufalino & Mayden 2010a,b,c) (Fig. 1). Phylogenetic analysis of morphological characters by Coburn and Cavender (1992) divided all phoxinins (including holarctic *Phoxinus*, some Asian genera and all Nearctic cyprinids, except *Notemigonus*) into three clades: 1) Western Clade sister to a 2) Chub Clade; and this clade sister to 3) Shiner Clade. This Western Clade consisted of 22 western genera, including species of the widespread *Rhinichthys* and monotypic *Tiaroga* (but excluded the western genera *Richardsonius* and *Oregonichthys*). A problem that plagued most of these studies was that many of the relationships of species and supraspecific taxa

were simply unresolved. The Western Clade included a large *Gila* Clade (13 genera); and its continued recognition as separate some of the 12 other genera in the clade rendered *Gila*, two decades from this writing, as a clearly unnatural grouping (para- or polyphyletic).

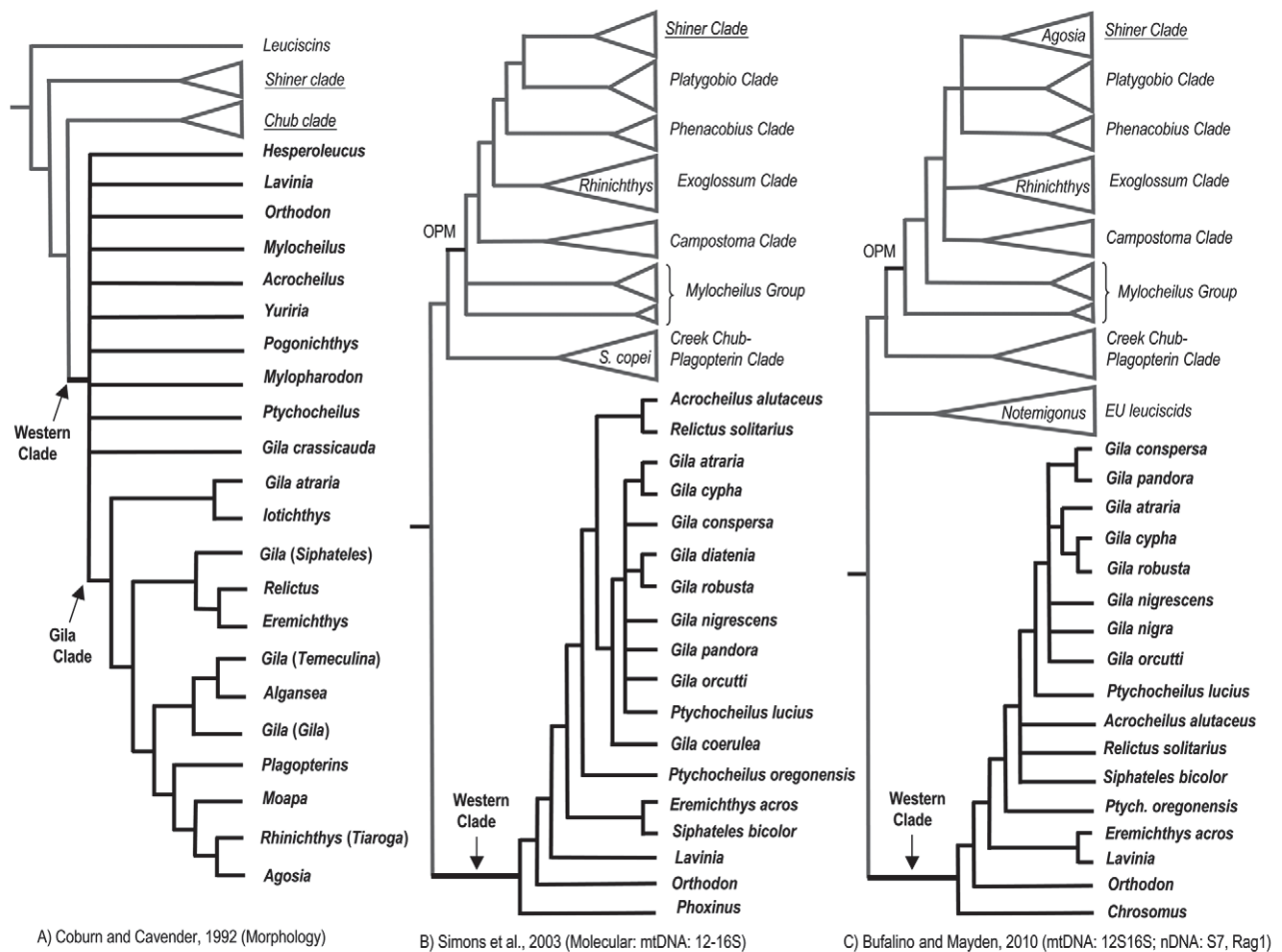


FIGURE 1. Previous phylogenetic hypotheses for the Western Clade based on morphological data: (A) osteological and lepidological characters (Coburn & Cavender 1992); and molecular data: (B) mitochondrial (12S–16S) DNA sequences (Simons *et al.* 2003); (C) mitochondrial (12S–16S) and nuclear (S7 and Rag1) DNA sequences (Bufalino & Mayden 2010). OPM: Open Posterior Myodome Clade.

Later phylogenies for North American cyprinids were based on mitochondrial sequences (Simons & Mayden 1997, 1998, 1999; Simons *et al.* 2003), and more recent analyses included nuclear genes (Bufalino & Mayden 2010a,b,c). These studies, like the earlier morphological studies also identified three major clades: 1) Western Clade, 2) Creek Chub-Plagopterin Clade (hereafter the CC- Plagopterin Clade) and 3) Open Posterior Myodome (OPM) Clade (Mayden's initial 1989 hypothesis corroborated in Simons & Mayden 1997, 1998, 1999; Simons *et al.* 2003). While a western clade was recognized in both morphological and molecular studies the latter proposed alternative relationships among the genera (Fig. 1); however, these studies only sampled 8 to 12 of the 22 genera of the Western Clade as proposed by Coburn and Cavender (1992). In fact, all previous studies based on either morphological or molecular data, failed to sample the needed array of intra-generic variation and can thus be considered inadequate due simply from taxon sampling error. Sampling of incomplete taxon coverage and limited characters can profoundly impact resulting phylogenies (Hillis 1998; Zwickle and Hillis 2002; Pollock *et al.* 2002; Hillis *et al.* 2003; Hedtke *et al.* 2006; Heath *et al.* 2008; Mayden *et al.* 2008, 2009; Mayden and Chen 2010). We hypothesize that this incomplete evaluation of the fauna in a broader context influenced resulting inferences as to relationships of the highly heterogeneous western cyprinid fauna.

Some western genera (i.e. *Ptychocheilus*, *Algansea*, *Gila*) include species that present a wide range of body sizes and forms and inhabit a wide variety of habitats. *Ptychocheilus* was proposed as a monophyletic group closely related to *Mylopharodon* based on morphological analyses (Carney & Page 1990; Mayden *et al.* 1991). A recent molecular analysis by Houston *et al.* (2010a), based on limited mitochondrial sequences, while not well supported,

did not resolve *Ptychocheilus* as monophyletic; *P. lucius* was more closely related to some species of *Gila* than to other species of *Ptychocheilus*. Conversely, molecular analyses of the heterogeneous genus *Algansea* have supported prior morphologic analyses by Barbour and Miller (1978) in identifying the genus as monophyletic, and resolved it sister to *Agosia*, both part of a clade including south western North American cyprinids (i.e. *Tampichthys*, *Codoma*, *Cyprinella*, *Yuriria*, *Hybognathus*, and Mexican *Notropis* (Schönhuth *et al.* 2008; Perez-Rodriguez *et al.* 2009). To date, no evidence exists as to the monophyly of *Gila*. Studies to date concluded that some *Gila* are more closely related to other genera than to other species of *Gila* (Coburn & Cavender 1992; Houston *et al.* 2010a) or that the closest relatives of *Gila* remain uncertain (Smith *et al.* 2002).

Material and methods

Our analysis included 127 specimens representing 71 species (including *Phoxinus* plus *Notemigonus*), from 122 localities. Of these specimens, 109 (54 species, from 103 localities) were from 22 genera included in Coburn and Cavender's Western clade. We made additional effort to include most of the species for all western genera, especially the heterogeneous western genera *Algansea*, *Gila*, *Lepidomeda*, *Ptychocheilus*, and *Siphateles*. Furthermore, 112 of the specimens (56 species; from 106 localities) represented western North American cyprinids. An additional 12 specimens (10 species, 12 localities) were of nonwestern North American cyprinids that were hypothesized by Coburn and Cavender (1992) to be part of the two other major phoxinin clades (Chub and Shiner clades) (*Chrosomus*, *Clinostomus*, *Couesius*, *Erymonax*, *Exoglossum*, *Hybopsis*, *Margariscus*, *Oregonichthys*, *Platygobio*, and *Richardsonius*). Two species (2 specimens) from 2 localities represented Far eastern cyprinids. Sequences from four species of *Couesius*, *Chrosomus* and *Platygobio* from North America, and *Tribolodon* from Asia, were not available for study and were obtained from GenBank (Appendix 1).

Voucher materials for most specimens are deposited in ichthyological collections. Institutional acronyms follow those of Leviton *et al.* (1985), as later modified by Leviton and Gibbs (1988), except for SLUM (Saint Louis University Fish Collection Museum, St. Louis, Missouri, USA) (Appendix 1).

One mitochondrial (mtDNA) and three nuclear (nDNA) regions were characterized from the same specimens. MtDNA was represented by complete sequences for cytochrome *b* (*cytb*, 1140 bp, as described by Schönhuth and Doadrio 2003), whereas variation in the nuclear genome was characterized using sequences from S7 ribosomal protein (*S7*, 1020 bp, including the first intron, Chow and Hazama 1998), recombinant activation gene 1 (*Rag1*, 1518 bp from exon 3, Lopez *et al.* 2004) and rhodopsin (*Rhod*, fragment of 843 bp, Chen *et al.* 2003). DNA extraction from tissue samples was performed using Dneasy Tissue extraction Kits (Qiagen, Valencia, CA, USA). All PCR amplifications were conducted in 50 microliter (μl) reactions. When necessary, nested PCR was performed for the S7 region with two internal primers (*S72-F*, Schönhuth *et al.* 2011 and *S72-R2* 5'-TCG CAC TGG TAC TGA ACA T-3') as described in Schönhuth *et al.* (2011). When more than one band occurred in the S7 amplifications, DNA was extracted from the gel (using DNA Gel Extraction kit, Qiagen, Valencia, CA, USA). Primers for direct sequencing of the purified PCR were the same as those used for PCR amplification. Purified PCR products were sent to University of Washington High-Throughput Genomics Unit (Seattle, WA, USA) for sequencing. Sequences specifically obtained for this study have been deposited in GenBank under accession numbers JX442981 to JX443417.

Cytb sequences obtained in this study were combined with previously published sequences. Sequences of each of the four regions were aligned manually with outgroup species from the Eurasian *Phoxinus phoxinus* and the North American *Notemigonus crysoleucas*. No ambiguous alignments or gaps were found in *cytb*, *Rag1* and *Rhod*. Nuclear S7 sequences were aligned using Clustal X ver1.85 (Thompson *et al.* 1997) and corrected to minimize substitutional changes. Multiple indels (ranging from 1 to 38 bp in length) were detected for S7, where sequence sizes of this region for all Cyprinidae examined ranged from 776 to 919 bp. No characters were excluded from analyses.

Sequences were analyzed in three different data sets (one for *cytb*, one for the combined three nuclear regions and one for all four DNA regions concatenated). Observed genetic divergences are presented as uncorrected *p*-distances (Table 1). Phylogenetic trees were estimated for each of the three data sets using Maximum Likelihood (ML) and Bayesian Inference (BI). ML trees were estimated using RAxML (Randomized Axelerated Maximum Likelihood, version 7.0.4, Stamatakis 2006). The search for optimal ML trees and bootstrap support was performed

on a high-performance iDiscover cluster computing facility (32 nodes) located at Saint Louis University. For the ML search with the mixed model of nucleotide substitution, we used GTR+I+G model (with four discrete rate categories). Inferences included partitions with respect to each gene. The ML tree search was conducted by performing 100 distinct runs using the default algorithm of the program for random trees (-d option) to obtain four starting trees, one for each run. The final tree was determined by a comparison of likelihood scores under the GTR+I+G model among suboptimal trees obtained per run. Robustness of the inferred tree was evaluated using bootstrap analysis on 1,000 pseudoreplications using RAxML 7.0.4 (Felsenstein 1985; Stamatakis *et al.* 2008). Resulting trees were imported into PAUP*4.0.b10 (Swofford 2001) to obtain the consensus tree. BI analyses were conducted for each of the three data sets using Mr. Bayes v3.1.2 (Huelsenbeck & Ronquist 2001). The Akaike information criterion (AIC) implemented in MODELTEST v3.4 (Posada & Crandall 1998) was used to choose an evolutionary model for each gene and used on the partitions for BI. For BI, 2,000,000 generations were implemented, sampling the Markov chain at intervals of 100 generations. A total of 1,000 trees (i.e., from the first 100,000 generations) were discarded as “burn-in.” Support for BI tree nodes was determined from Bayesian posterior probabilities obtained from a majority-rule consensus tree with PAUP* (Swofford 2001).

ML trees inferred from each data set, *cytb* (1140 bp), nuclear data (3395 bp), and all concatenated sequences (4536 bp), are represented with ML bootstrap (BS) support, followed by posterior probabilities (PP) (Figs. 2, 3).

Results

MtDNA and nDNA phylogenies inferred using Maximum likelihood and Bayesian analyses were largely congruent, yielding three distinct and well-supported clades (Figs. 2, 3) (i.e., ML BS > 70% and Bayesian PP > 85%) in all analyses (Table 1). The 55 species corresponding to 22 North American genera of the Western Clade *sensu* Coburn and Cavender's (1992), were always resolved within these three divergent major clades. Phylogenetic relationships between these three distinct clades were not resolved in any analyses.

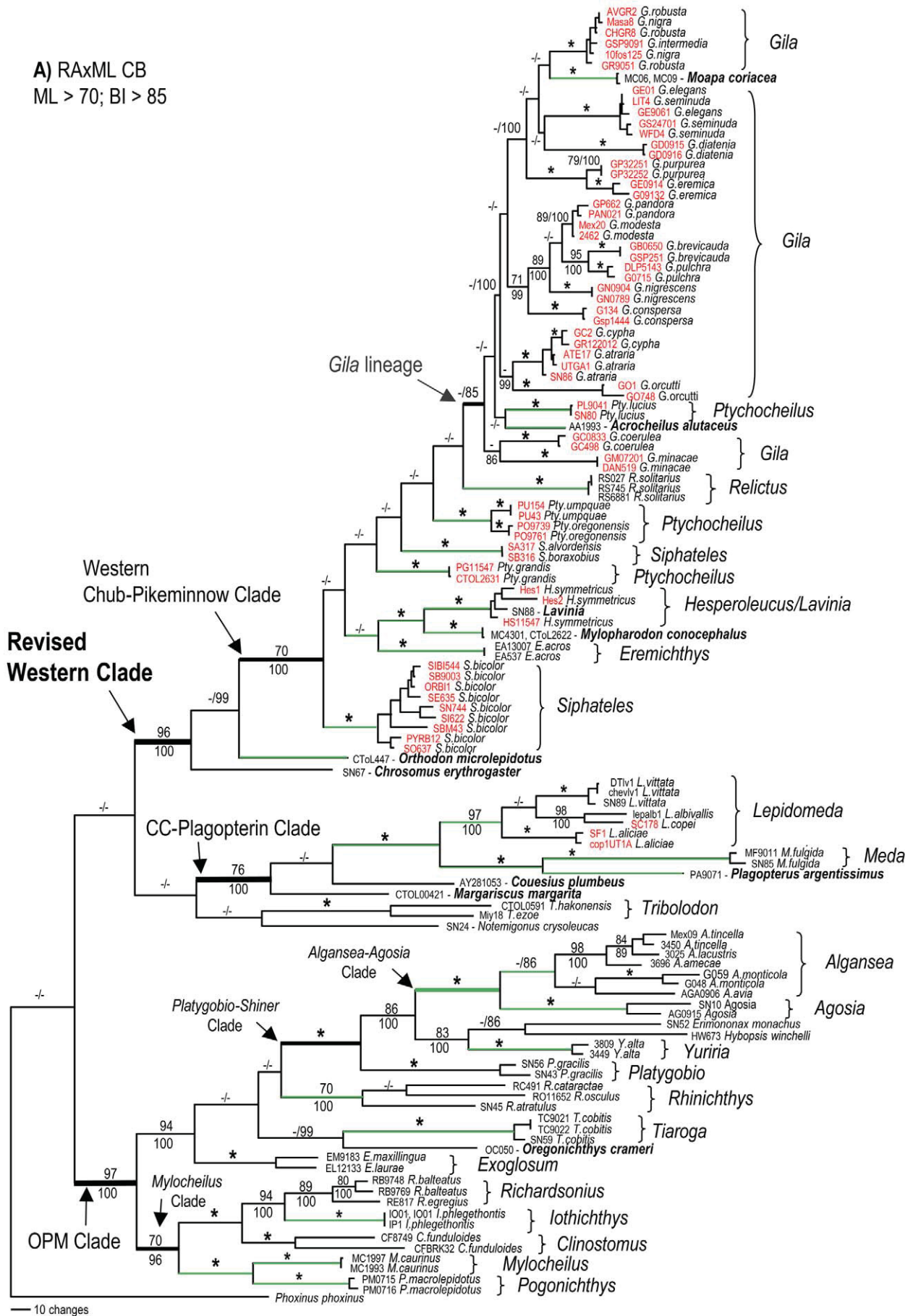
All analyses resolved a well-supported clade inclusive of 35 species from twelve widely distributed and mostly western genera (hereafter referred to as the revised Western Clade, RWC). This clade included 11 western genera (*Acrocheilus*, *Eremichthys*, *Gila*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, *Orthodon*, *Ptychocheilus*, *Relictus* and *Siphateles*) plus *Chrosomus*. Within the RWC, *Chrosomus erythrogaster* and *Orthodon microcephalus* were always basal to the remaining 33 species from ten genera, forming the well-supported and heterogeneous Western Chub-Pikeminnow Clade. MtDNA variation and the following analyses provided longer terminal branches and more resolution at terminal nodes than did nDNA within this clade. All analyses included *Lavinia*, *Hesperoleucus* and *Mylopharodon* in a well-supported monophyletic group within the Western Chub-Pikeminnow Clade; however, relationships between *Acrocheilus*, *Eremichthys*, *Gila*, *Moapa*, *Ptychocheilus*, *Relictus* and *Siphateles* were not well resolved or supported (Figs. 2, 3). Phylogenetically informative variation of both sets of genes resolved nearly all species as reciprocally monophyletic (Figs. 2A, 2B).

The second major clade included 15 genera occupying a wide geographic range, only eight of which were from Coburn and Cavender's Western Clade. These included *Agosia*, *Algansea*, *Iothichthys*, *Mylocheilus*, *Pogonichthys*, *Rhinichthys*, *Tiaroga* and *Yuriria*. This clade also included seven genera from Coburn and Cavender's Shiner and Chub clades, including *Clinostomus*, *Erimonax*, *Exoglossum*, *Hybopsis*, *Oregonichthys*, *Platygobio* and *Richardsonius*. Together these genera formed a clade identified earlier by Bufalino and Mayden (2010a, b, c) as the open posterior myodome, or OPM, clade.

Within the OPM clade, three western genera (*Pogonichthys*, *Mylocheilus* and *Iothichthys*), considered by Coburn and Cavender to be part of their Western Clade, were resolved within a monophyletic group inclusive of *Richardsonius* and *Clinostomus*. *Iothichthys* was always sister to *Richardsonius* as in previous mtDNA analyses (Smith *et al.* 2002; Houston *et al.* 2010b), and together this clade was sister to *Clinostomus* (Estabrook *et al.* 2007; Houston *et al.* 2010b). *Mylocheilus* plus *Pogonichthys* formed the sister group to the former clade.

Other western minnows in the OPM clade include *Agosia*, *Algansea*, *Rhinichthys*, *Tiaroga* and *Yuriria*. *Agosia* and *Algansea* are part of a clade that was always inferred to be closely related to some shiners (*Hybopsis*, *Erimonax*), and with Mexican *Yuriria* and *Platygobio* from the Shiner-*Platygobio* Clade. *Rhinichthys* and *Tiaroga* were identified as divergent lineages that were part of a polytomy making each potential sister lineages to the Shiner-*Platygobio* Clade.

A) RAXML CB
ML > 70; BI > 85



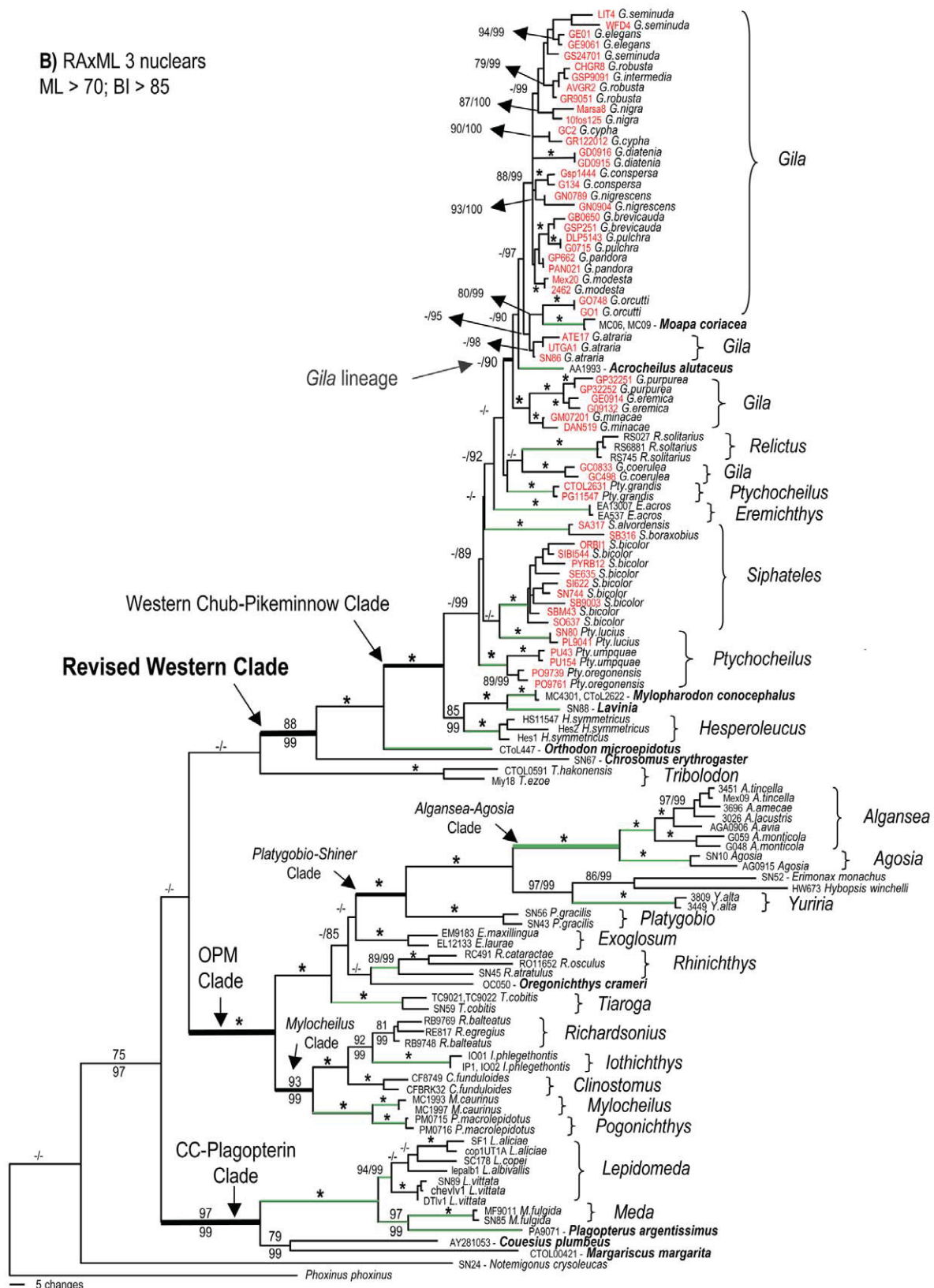


FIGURE 2. Phylogenetic relationships of all specimens analyzed according to sequence variation across (A) the cytochrome *b* mitochondrial gene; (B) the three combined nuclear regions (*Rag1*, *S7* and *Rhod*); best RAxML tree using GTR+I+G model. Numbers on branches are ML bootstrap support (BS > 70%) and Bayesian posterior probabilities (PP > 85%). Asterisks are given when all values were 100%; “-” are given when BS and / or PP values were less than those listed above. In “red” are those taxa recovered from nonmonophyletic genera. In “green” are those nodes from genera (other than *Gila*) comprising Coburn and Cavender’s (1992) “Western Clade”.

RAxML 4 genes
ML > 70; BI > 85

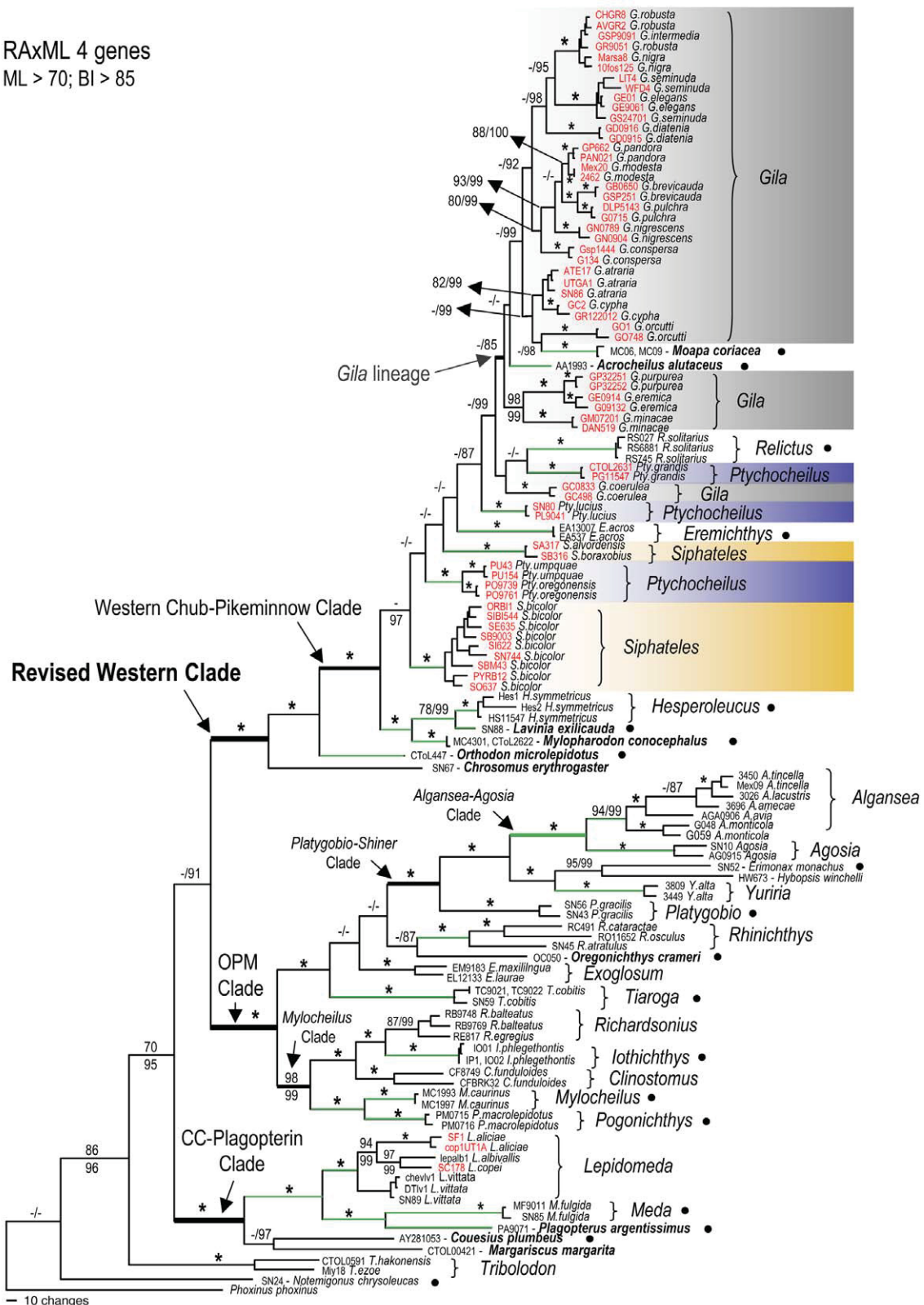


FIGURE 3. Phylogenetic relationships of all specimens analyzed according to sequence variation across combined mitochondrial and nuclear regions (*cytb*, *Rag1*, *S7* and *Rhod*); best RAxML tree using GTR+I+G model. Numbers on the branches are ML bootstrap support (BS > 70%) and Bayesian posterior probabilities (PP > 85%). Asterisks are given when all values were 100%; “-” are given when BS and / or PP values were less than those listed above. In “red” are those taxa recovered from nonmonophyletic genera. In “green” are those nodes from genera (other than *Gila*) comprising Coburn and Cavender’s (1992) “Western Clade”. Black dot after species name indicate those monotypic genera. Shading indicated those genera recovered as nonmonophyletic.

The third major clade was composed of genera comprising the Creek Chub and Plagopterin Clades (CC-Plagopterin Clade) following Simons and Mayden (1997) and Simons et al. (2003). This well-supported group included a lineage inclusive of all of the Plagopterin genera, *Lepidomeda*, *Meda* and *Plagopterus*. Analyses of nuclear and mtDNA sequences always placed *Snyderichthys* (formerly *Gila copei*) within *Lepidomeda*, and this clade was sister to *Meda* plus *Plagopterus*, all together forming the well-supported Plagopterin Clade of Simons and Mayden (1997) and Dowling et al. (2002). This Plagopterin Clade was always resolved sister to *Margariscus* and *Couesius*, and while not all of their relationships could be resolved, they formed the well-supported CC-Plagopterin Clade.

Discussion

Analyses of sequences from one mtDNA gene and three nuclear genes yielded valuable information relative to hypothesized relationships among western North America cyprinids. Coburn and Cavender (1992) were the first to conduct a formal, all-inclusive analysis of cyprinids from western North America, providing direction for recent molecular studies of this group (e.g., Simons & Mayden 1997, 1998; Dowling et al. 2002; Simons et al. 2003; Bufalino & Mayden 2010a,b,c).

The Western Clade of Coburn and Cavender (1992) included 11 genera that are resolved as part of our RWC. However, the former authors also included several other genera from western North America in their inferred clade (*Agosia*, *Algansea*, *Iotichthys*, *Mylocheilus*, *Pogonichthys*, *Rhinichthys*, *Tiaroga*, *Yuriria*, and plagopterins [including *Plagopterus*, *Meda*, *Lepidomeda*]). Our analyses resulted in RWC wherein these latter genera were not members included, but were part of other, well supported, OPM and CC-Plagopterin major lineages (Figs. 2A, 2B, 3). These results are in general agreement with prior molecular studies that included fewer representatives of western North American genera based on mtDNA (Simons & Mayden 1997, 1998, 1999; Simons et al. 2003; Ruber et al. 2007; Houston et al. 2010b), or also including nDNA (Bufalino & Mayden 2010a,b,c). The Western Clade *sensu* Coburn and Cavender (1992) was not corroborated as a monophyletic group.

Western cyprinids in the OPM clade

Eight genera from Coburn and Cavender's Western Clade (*Agosia*, *Algansea*, *Iotichthys*, *Mylocheilus*, *Pogonichthys*, *Rhinichthys*, *Tiaroga*, *Yuriria*) were resolved as descendants of the widely-distributed, well-supported clade that also included seven genera from Coburn and Cavender's Shiner and Chub clades (*Clinostomus*, *Erimonax*, *Exoglossum*, *Hybopsis*, *Oregonichthys*, *Platygobio*, *Richardsonius*), a group previously recognized by Bufalino and Mayden (2010a,b,c) as the Open Posterior Myodome Clade (OPM) clade. The OPM clade is resolved as two well-supported lineages that render western genera as an unnatural (para-polyphyletic) group within this clade (Figs. 2A, 2B, 3).

Within the OPM clade, three western genera (*Iotichthys*, *Mylocheilus* and *Pogonichthys*), formerly in Coburn and Cavender's Western Clade, formed a clade with *Richardsonius* and *Clinostomus*, a relationship also resolved in Simons & Mayden (1999) and Estabrook et al. (2007). Even though this clade, previously identified as the *Mylocheilus* Clade, was not always supported in prior molecular studies (Simons et al. 2003; Bufalino & Mayden 2010b,c), it was a well-supported group in our mtDNA and nDNA analyses. The monotypic genus *Iotichthys* was hypothesized by Coburn and Cavender (1992) to be closely related to *Gila atraria* in their Western Clade, while *Richardsonius* (including *Clinostomus*) was hypothesized to be sister to *Gila* by Uyeno (1960). Here, *Iotichthys* was always resolved as sister to *Richardsonius* as in previous mtDNA analyses (Smith et al. 2002; Houston et al. 2010b), and this clade was sister to *Clinostomus* (Estabrook et al. 2007; Houston et al. 2010b). The clade *Mylocheilus* plus *Pogonichthys* was the sister group to the above clade (e.g., ((*Mylocheilus* + *Pogonichthys*)(*Iotichthys* + *Richardsonius*) *Clinostomus*)).

While Coburn and Cavender (1992) included *Agosia* and *Rhinichthys* in a *Gila* clade within their Western Clade, they also noted *Rhinichthys* and *Agosia* were excluded from this clade when scale characters were removed, a phylogenetic resolution supported by recent molecular studies (Dowling et al. 2002; Simons et al. 2003). Similarly, when the Asian taxa *Tribolodon* and *Rhychoypris* were removed from Coburn and Cavender's analysis, resulted in the Chub Clade embedded deeply within the Western Clade in a trichotomy with *Rhinichthys* and

Agosia. The position of the Asian genus *Tribolodon* is not resolved in our study with increased samples and characters in separated analyses from mtDNA and nDNA genes. Rather, *Tribolodon* is a basal group to the three major clades in the combined analyses.

Algansea, endemic to central Mexico, has also previously been identified as closely related to some species of *Gila* (Barbour & Miller 1978; Coburn & Cavender 1992). Contrary to these morphology-based studies, prior molecular analyses resolved this genus in a sister-group relationship with *Agosia* (Schönhuth *et al.* 2008). This well-supported clade was again identified herein; however, this clade was not resolved closely related to either *Gila* or *Rhinichthys* as hypothesized in prior morphological-based studies (Barbour & Miller 1978; Woodman 1992, respectively). Rather, this clade was a member of the Shiner-*Platygobio* Clade within the OPM clade, and was never closely related to any species of our RWC.

The phylogenetic resolution of *Rhinichthys* and *Tiaroga* herein is also inconsistent with previous morphological studies (Woodman 1992; Coburn & Cavender 1992) wherein *Tiaroga* was nested within *Rhinichthys* and both genera were considered members of the Western Clade. Herein, the monotypic genus *Tiaroga* was found to be highly divergent and not immersed within the widely distributed genus *Rhinichthys*, in agreement with a prior mtDNA analysis by Simons & Mayden (1999), and neither genus was resolved as part of the Western Clade *sensu* Coburn & Cavender. Rather, these genera were both within our recognized OPM Clade.

Western cyprinids in the CC-Plagopterin clade

Uyeno's (1960) comparative osteological study of *Gila* and relatives resolved *Snyderichthys* and *Siphateles* as subgenera within *Gila*; *Clinostomus* plus *Richardsonius* formed the sister group to *Gila*. Coburn and Cavender (1992) likewise included the monotypic genus *Snyderichthys* (Miller 1945b) and *Siphateles* within *Gila*. More recent molecular analyses based on mitochondrial genes could not corroborate these relationships; *Snyderichthys* was resolved as more closely related to *Lepidomeda* than to any species currently or then referred to *Gila* (Simons & Mayden 1997; Dowling *et al.* 2002; Simons *et al.* 2003). In fact, this is in agreement with close morphological resemblance, and possible close relationships, as mentioned by Miller & Hubbs (1960). Here, analyses of nuclear and mtDNA sequences always recovered *Lepidomeda*, *Meda*, *Plagopterus*, and *Snyderichthys* in the well-supported Plagopterin Clade (*sensu* Simons & Mayden 1997; Dowling *et al.* 2002), and as part of the well-supported CC-Plagopterin Clade. These overall relationships are in agreement with phylogenetic hypotheses by earlier systematists (Simons & Mayden 1997; Dowling *et al.* 2002; Simons *et al.* 2003; Johnson *et al.* 2004). Our results were also consistent with previous analyses of the divergences in the *Snyderichthys copei* (leatherside chub). This "species" was resolved as two separate and highly divergent lineages (*cytb* divergences 7.8–7.9%). Thus, this "species" is resolved paraphyletic with regard to *Lepidomeda vittata* and *L. albivallis*, consistent with the recognition of the northern leatherside chub (*Lepidomeda copei*) and the southern leatherside chub (*Lepidomeda aliciae*) as distinct species (Dowling *et al.* 2002; Johnson *et al.* 2004).

Western Clade

The remaining 34 species from 11 genera from western North America (*Acrocheilus*, *Eremichthys*, *Gila*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, *Orthodon*, *Ptychocheilus*, *Relictus* and *Siphateles*) are included in our RWC; this portion of the overall phylogeny is consistent with the results of Coburn and Cavender (1992). Of special interest, however, is the position of the genus *Chrosomus* within this clade. Members of this genus have been thought to be closely related to the Holarctic genus *Phoxinus* (Banareescu 1964; Howes 1985). In Coburn & Cavender's (1992) morphological analyses, *Chrosomus* (these authors used North American representatives and referred to them as *Phoxinus*; *Phoxinus* is now restricted to Eurasia *sensu* Strange & Mayden (2009) was always recovered in a basal position in their Chub Clade. Herein, *Chrosomus* was always resolved in a basal position within our RWC. This finding is in agreement with other recent molecular phylogenies by Simons *et al.* (2003) and (Bufalino & Mayden 2010a, b, c), and not closely related to *Phoxinus* as referenced above.

An additional case of note is the relationship of *Hesperoleucus* and *Lavinia*. Our analyses resolve the mtDNA gene lineage of *Hesperoleucus symmetricus* as being both closely related and paraphyletic with respect to *Lavinia* (*cytb* divergences between both species: 0.8–2.3%). However, the nDNA gene lineages clearly identify

divergences between these two species, and recover *Lavinia* as both highly differentiated from and sister to *Mylopharodon*. These two species as a lineage are sister to *Hesperoleucus*. Morphological and ecological differences between *Lavinia exilicauda* and *Hesperoleucus symmetricus* are considerable, leading to their original placement in separate genera (Miller 1945a; Avise *et al.* 1975), representing another example of degree of divergence interpreted as taxonomic rank at that time. Some studies have concluded that these species hybridize and there is evidence of introgression in sympatric populations (Miller 1945a; Aguilar & Jones 2009). Both species have been shown to be similar based on allozymes (Avise *et al.* 1975), as well as microsatellites and mtDNA variation (Aguilar & Jones 2009). In the latter study, mtDNA recovered some highly divergent populations within the range of *Hesperoleucus* but Aguilar and Jones (2009) did not recognize the two species as independent lineages and *Hesperoleucus* was referred to as *Lavinia* (Brown *et al.* 1992; Jones *et al.* 2002; Aguilar & Jones 2009). The conflict between the phylogenetic interpretation of variation in mtDNA and nDNA genes in this situation may be indicative of incomplete lineage sorting during the divergence of not only these two species but others (*Mylopharodon*) or post-divergence introgression (Aguilar & Jones 2009). Given the stark differences between mtDNA and nDNA gene lineages in this particular case warrants further, more fine-scale analyses, using varied methods evaluating genetic variability across the ranges of these species before any taxonomic decisions are finalized (Figs. 2, 3).

Some current genera resolved within the heterogeneous Western Chub-Pikeminnow Clade (i.e. *Gila*, *Mylopharodon*, *Ptychocheilus* and *Siphateles*) appear to be old inhabitants of western North America as evidenced by fossil records of these taxa dating from middle Miocene in the Great Basin (Smith *et al.* 2002). Current high levels of morphological diversity within this Western Chub-Pikeminnow Clade may support a long independent evolutionary history of each lineage. However, this is not consistent with the historically promulgated believe that genetic divergence is tightly correlated morphological divergence, or age since origin. Relatively low levels of genetic divergence were observed within and between these genera of this clade. Inter-generic mtDNA divergences estimated within this heterogeneous Western Chub-Pikeminnow Clade ranged from 4.8–7.3% (*Acrocheilus*-*Gila*) to 11.5% (*Moapa*-*Hesperoleucus*). Smith *et al.* (2002) did hypothesize that diversity in Great Basin fishes over geologic time could have been impacted by cyclical isolation (during interglacial periods) that may have resulted in periods of secondary contact, thus both increasing extinction rates of lineages and exchange of genes during periods when species were in contact (or hybridization), a common phenomenon in cyprinids (Hubbs 1955). Cyclical changes in abiotic and biotic factors could have led to high extinction rates (Smith *et al.* 2002) and facilitated the transfer of genetic variability (Arnold 1997; Dowling & Secor 1997) that may have accumulated in isolated lineages through hybridization under this theory.

Regardless of the mechanism, the clearly documented morphological heterogeneity in this group is not always correlated with observed genetic divergence. For example, levels of inter-generic *cytb* divergences can be highly variable as demonstrated by pairwise comparisons among the seemingly morphologically divergent genera (i.e.: *Acrocheilus*-*Relictus*: 8.1; *Moapa*-*Gila*: 5.1–8.7%; *Hesperoleucus*-*Mylopharodon*: 6.0–6.1%) and within them (i.e., *Gila*: 0.2–9.6%; *Ptychocheilus*: 1.7–8.0%; *Siphateles*: 0.2–9.8%). Hence, genetic and morphological anagenesis are inconsistent between and within these genera and are not positively correlated with time. Our inferred phylogenetic relationships of species/genera from all of our analyses refuted previously hypothesized ideas of a monophyletic nature of *Gila*, *Siphateles* and *Ptychocheilus* (Table 1, Figs. 1, 3). This failure to resolve these genera as monophyletic, in itself, is likely a large part of the inconsistencies between morphological and molecular divergences as relevant comparisons cannot be made between artificially grouped taxa into genera. The 19 species currently included in *Gila* the three species of the genus *Siphateles*, and the four species from the genus *Ptychocheilus* were always recovered nested with seven other monotypic genera within the Western Chub-Pikeminnow Clade (Figs. 1, 3) (*Gila*: *G. atraria*, *G. breviceauda*, *G. coerulea*, *G. conspersa*, *G. cypha*, *G. diatenia*, *G. elegans*, *G. eremica*, *G. intermedia*, *G. minacae*, *G. modesta*, *G. nigra*, *G. nigrescens*, *G. orcutti*, *G. pandora*, *G. pulchra*, *G. purpurea*, *G. robusta*, and *G. seminuda*; *Siphateles*: *S. bicolor*, *S. alvordensis* and *S. boraxobius*; *Ptychocheilus*: *P. lucius*, *P. grandis*, *P. oregonensis*, and *P. umpqua*). Because resolution of nodes defining these genera is limited, further investigation using other mtDNA and nDNA genes is necessary to gain a better understanding of and stronger support for relationships before any taxonomic changes are recommended. However, all of these species should be annotated in their classification, along with the seven monotypic genera as “incertae sedis” *sensu* Wiley’s (1981) recommendation for cases where relationships are not fully understood or supported.

Within the Western Chub-Pikeminnow Clade, species of *Gila* were recovered in a well-supported lineage with two monotypic genera - *Moapa* and *Acrocheilus*, in all data sets with Bayesian analyses. Hereafter, this clade is referred to as the *Gila* lineage to differentiate this natural group from the traditional genus *Gila*. However, composition of this *Gila* lineage is slightly different in mtDNA and nDNA analyses, as the inclusions of either *Ptychocheilus lucius* or *Gila coerulea* in the *Gila* lineage was not well-supported; these taxa were resolved within the *Gila* lineage in mtDNA analyses, but were excluded from this lineage in all analyses using either nDNA and the analyses using the combined mtDNA and nDNA data (Figs. 2, 3). Thus, the *Gila* lineage resulted in a widespread morphologically diverse group of 22 or 20 species (in mtDNA or nDNA analyses, respectively) from different genera, all inhabiting distant, isolated drainages across western North America. This lineage displays a wide range of inter-specific *cytb* variation (0.2–9.6%) relative to the equally diverse range recovered for the genus *Algansea* (2.1–9.3%), and was unexpectedly lower in comparison with *cytb* variation estimated for monophyletic genera within Shiners or Chubs from eastern North America (*Luxilus*: 8.5–11.4%; *Campostoma*: 2.9–10.7%, *Nocomis*: 4.0–14.5%) (Schönhuth *et al.* 2008; Schönhuth & Mayden 2010). These eastern taxa inhabit more continuous, high-gradient, and significantly more permanent flowing aquatic habitats, while at the same time displaying less morphological heterogeneity.

Within the morphologically heterogeneous *Gila* lineage some species now considered to be more distantly related to one another than they are to other species of *Gila* exhibit similar morphological features consistent with the hypothesis that taxa in similar selective environments may converge on similar forms. Morphological characteristics that tend to be affected (e.g., streamlined body forms, flattened head, humped dorsal surfaces, leathery skins with fine or embedded scales, and large falcate fins) could be adaptations to fast flowing rivers as has been demonstrated in the lake whitefish *Coregonus clupeaformis* (Bernatchez & Dodson 1990; Lu & Bernatchez 1999), threespine stickleback *Gasterosteus aculeatus* (Orti *et al.* 1994; Schluter *et al.* 2004; Colosimo *et al.* 2005), and members of the genus *Gila* (Smith *et al.* 1979). These may have evolved in, and remain in, a selective regime that over evolutionary time has constrained them to a more limited morpho-space. This may also be true for some recent molecular phylogenetic studies recovering recognized genera as paraphyletic, and with cryptic species diversity in some genera. In these cases our classification of these genera required fundamental taxonomic revisions to maintain consistency between phylogenetic relationships and a natural classification (e.g., Dowling *et al.* 2002; Johnson *et al.* 2004; Schönhuth *et al.* 2008). Conversely, accumulated morphological divergences that can be identified within this Western Chub-Pikeminnow Clade could be influenced more by natural selection in local environments than by time. In all of this nature one is faced with incongruences to establishing a natural classification if one considers comparative overall morphological similarity between taxa relative to inferred genealogical relationships that do not consider such similarities as evidence but are based solely on shared derived characters—synapomorphies. Thus, while not evaluated quantitatively in a formal comparative analysis, the morphological differences observed in this group may not be consistent with observed genetic divergences within this lineage.

On the basis of the above considerations, we suggest retaining the names for most of the western monotypic genera included in the Western Chub-Pikeminnow Clade (*Eremichthys*, *Relictus*, *Hesperoleucus*, *Mylopharodon*, *Lavinia*). However, some comments below apply for certain lineages presently included in the nonmonophyletic genera within this clade. Within the *Gila* lineage, all analyses consistently nested the monotypic genera *Moapa* and *Acrocheilus* within *Gila*. MtDNA divergences between species of *Gila* ranged between 0.2–9.6%, similar to divergences for these monotypic genera and species of *Gila* (*Acrocheilus*-*Gila*: 4.9–7.8%, and *Moapa*-*Gila*: 4.7–8.8%). *Ptychocheilus lucius* is recovered within the *Gila* lineage but only in mtDNA analyses, while *Gila coerulea* (*Klamathella* by Smith *et al.* 2002) is excluded from this lineage in nDNA analyses and in the combined data set. A more detailed study is warranted wherein the present data are combined with both more conservative (for more basal lineage resolution) and more highly variable genes (for resolving relations within and among species) than used herein. We also recommend increased taxon and population sampling of the highly heterogeneous *Gila* lineage. This type of analysis and one combined with existing and new morphological data (osteological and other characters) may provide additional insight into relationships and the potential impacts of formerly hypothesized introgression among these taxa. We provisionally consider all species within the *Gila* lineage as *Gila* (including *Moapa coriacea* and *Acrocheilus alutaceus*) pending a more comprehensive analysis for this heterogeneous group; this will be treated in the comprehensive revision of the genus *Gila* currently prepared by Susana Schonhuth and Richard L. Mayden.

The genus *Siphateles* was always recovered within the Western Chub-Pikeminnow Clade. *Siphateles* was recognized as a separate genus from *Gila* in prior molecular analyses based on the phylogenetic position of *S. bicolor* (Simons & Mayden 1998). The three currently recognized species in the genus showed divergences ranging from 0.2 to 9.8%. However, *Siphateles* was not recovered as a natural group in any of our analyses, contrary to prior molecular analyses (Harris 2000). Different forms analyzed here representing the *Siphateles bicolor* complex (divergences *cytb* 0.7–3.5%) were always recovered in a well-supported group, but were never recovered as sister to *S. alvordensis* and *S. boraxobius*. Divergences between the *Siphateles bicolor* complex and *S. alvordensis* - *S. boraxobius* (8.8–9.8%) were similar to those for *Siphateles* - *Gila* (6.8–11.0%), *Gila* - *Mylopharodon* (7.8–10.5%), *Gila* - *Hesperoleucus* (8.4–11.5%), *Gila* - *Relictus* (7.9–10.1%), and *Gila* - *Lavinia* (8.7–11.5%), and higher than those between *Lavinia* - *Mylopharodon* (5.8–6.0%) or *Gila* - *Ptychocheilus lucius* (5.2–7.7%).

The genus *Ptychocheilus* was always recovered within the Western Chub-Pikeminnow Clade. Prior analyses considered *Ptychocheilus* to be a natural group/genus and closely related to *Gila* (Uyeno 1960). Here, this genus was never supported as a monophyletic group, a result in agreement with prior molecular analyses (Simons *et al.* 2003; Buffalino & Mayden 2010a,b). *Ptychocheilus oregonensis* was always recovered sister to *P. umpquae*, in agreement with a recent mitochondrial phylogeny for this genus (Houston *et al.* 2010a); while *Ptychocheilus grandis* and *P. lucius* were recovered in two separate groups within the Western Chub-Pikeminnow Clade. Therefore, further study of additional loci and characters is necessary to resolve these relationships.

Conclusions

Our resulting phylogenetic analyses of mtDNA and nDNA variation in all extant western North America cyprinid genera, agreed with the morphology-based phylogeny of Coburn and Cavender (1992) only to the extent that this cyprinid fauna is not a monophyletic group. Phylogenetic analysis of mtDNA and nuclear gene variation supports a more restrictive concept of the “Western Clade” as proposed by previous authors to our RWC. The pattern and rate of nuclear and mtDNA sequence evolution was heterogeneous within all western genera analyzed, as is typical in other recent analyses for Cypriniformes, but the four different DNA regions in many areas yielded generally concordant topologies (Figs. 2, 3). Our study corroborates many of the previous findings of Simons and Mayden (1997, 1998), wherein these authors excluded the genera *Mylocheilus*, *Pogonichthys*, *Rhinichthys*, *Tiaroga*, and *Agosia* from the Coburn and Cavender’s Western Clade. Analysis presented here further resolves relationships and clearly supports the exclusion of the genera *Algansea*, *Iotichthys* and *Yuriria* from the Western Clade. Our revised Western Clade does not include the Plagopterins or Creek-Chub lineages (represented by *Lepidomeda* (including *Snyderichthys*), *Meda* and *Plagopterus* versus *Couesius* and *Margariscus*, respectively).

Our mitochondrial and nuclear data provide support for a revised monophyletic Western Clade grouping 35 recognized species from 12 recognized genera, including 11 western genera (*Acrocheilus*, *Eremichthys*, *Gila*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, *Orthodon*, *Ptychocheilus*, *Relictus* and *Siphateles*), sister to the primarily northerly distributed species of *Chrosomus*. Analyses provide strong support for an additional clade within our RWC, the Western Chub-Pikeminnow Clade, that contains all species of *Gila* plus nine other western genera (*Acrocheilus*, *Eremichthys*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, *Ptychocheilus*, *Relictus* and *Siphateles*).

Our Western Chub-Pikeminnow Clade does not reflect the current classification of North American cyprinids. Neither the 19 species of *Gila* nor the three species of *Siphateles* (*S. bicolor* complex, *S. alvordensis*, *S. boraxobius*) or the four species of *Ptychocheilus* (*P. lucius*, *P. grandis*, *P. oregonensis*, *P. umpquae*) were ever recovered as monophyletic groups, respectively. More detailed studies of these taxa are necessary and may warrant reallocation of constituent species to new genera. The *Gila* lineage recovered here, is not as heterogeneous as Coburn and Cavender (1992) proposed (including *Gila* plus 12 other genera), nor so little as Smith *et al.* (2002) indicated (including *Gila* and *Moapa*, but excluded *G. coerulea* and *Acrocheilus*). Rather, it is a lineage that includes all current species of *Gila* and as well as *Moapa* and *Acrocheilus*. The taxonomy of this *Gila* lineage clearly warrants additional study.

North American western genera: taxonomic revisions and further analyses

This study clearly identifies the need for focused studies of some western taxa within the Western Chub-Pikeminnow Clade. We retain all current generic names, but the classification of some genera and some western groups must be re-examined due to: (i) close relationships of species from seven western genera (*Acrocheilus*, *Eremichthys*, *Hesperoleucus*, *Lavinia*, *Moapa*, *Mylopharodon*, and *Relictus*) with species of *Gila*, *Siphateles* and *Ptychocheilus*; (ii) limited support for the monophyly of some genera within the well-supported Western Chub-Pikeminnow Clade, and with each having similar genetic distances between and within some recognized genera; and (iii) the high likelihood of the genera *Gila*, *Siphateles*, and *Ptychocheilus* being nonmonophyletic.

Several taxa are at issue. *Acrocheilus* and *Moapa* always occurred within a *Gila* lineage, rendering the genus as paraphyletic, but together they could represent the genus *Gila sensu stricto*. The phylogenetic position of *Gila coerulea* (*Klamathella* of Smith *et al.* 2002) is ambiguous depending upon the class of genes used, and is either within or outside of this lineage. Comprehensive and more detailed analyses for the genus *Siphateles* are warranted to determine if the genus should be restricted to the *S. bicolor* complex, while a new genus is used for *Siphateles alvordensis* and *S. boraxobius*. Finally, *Ptychocheilus* warrants further analyses involving additional characters that will hopefully resolve the phylogenetic relationships of the current species and develop a classification consistent of their genealogical relationships.

The limits of these genera have all been debated previously, but less so within the context of phylogenetic analysis of morphological and molecular variation. Undoubtedly the divisional arrangement of genera is tentative at this point and may undergo further modifications as new characters/analyses become available and additional taxa are included. However, it appears highly likely from this analysis that the use of additional taxa and genes changes the classification of western North American species.

Acknowledgments

We especially thank David L. Ward, Bernie R. Kuhajda, Casey B. Dillman, Hector Espinosa, Phillip M. Harris, Dennis M. Stone and David A. Neely for assistance with sampling specimens, and to all those that collaborated on different field trips or who provided samples. Our thanks are also extended to the Government of Mexico for collecting permits to Héctor Espinosa (Permiso de Pesca de Fomento 0765). This research was supported by postdoctoral fellowship from the Spanish Ministerio de Educación y Ciencia (EX-2005-0106 to S. Schönhuth), the USA National Science Foundation program (DEB-0240184, EF-0431326, DBI-0956370, and DEB-1021840 to R.L. Mayden), Saint Louis University, and the William S. Barnickle Endowment.

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Appendix 1: Species and sampling localities for the material analyzed. Collection numbers are listed for vouchers stored at institutional collections followed by tissue/DNA numbers. *Dr.: Drainage; Co.: county; USA: United States of America; MX: Mexico.*

A. American western cyprinid genera						
Species	River	Voucher (Tissue)	Rag -1	Rhod	S -7	Cyt b
<i>Acrocheilus alutaceus</i>	Kettle River below falls at Cascade, Columbia River Dr. British Columbia, Canada.	UAIC 11365.01 (AA1993)	AA1993	AA1993	AA1993	AA1993
<i>Algansee amecae</i>	Cañon Coronilla, Ameca Dr., Jalisco, MX.	(MNCN3696)	3696	3696	3696	3696
<i>Algansee avia</i>	Rio Compostela, Chila Dr., Nayarit, MX.	SLUM 1106 (AGA0906)	AGA0906	AGA0906	AGA0906	AGA0906
<i>Algansee lacustris</i>	Lago Pazticularo, Lerma Dr., Michoacan, MX.	(MNCN3026)	3026	3026	3026	3026
<i>Algansee monticola</i>	Rio Bolaños at Valparaíso 1.5 Km N Valparaíso, Santiago Dr., Zacatecas, MX.	UANL 18775 (G048)	G048	G048	G048	G048
	Arroyo s/n in Sauz de Abajo, Santiago Dr., Zacatecas, MX.	UANL 18875 (G059)	G059	G059	G059	G059
<i>Algansee tinicella</i>	Presa Orandino, Orandino, Lerma Dr., Michoacan, MX.	UAIC 15398.01 (MEX09)	MEX9	MEX9	MEX9	MEX09
	Laguna Zacapu, Panindicuaro, Lerma Dr., Michoacan, MX.	(MNCN3450-51)	3451	3451	3451	3450
<i>Agosia chrysogaster</i>	No locality data.	UAIC 13018.01	SN10	SN10	SN10	SN10
	Concepcion River, Bridge at Hwy 15, Sonora, MX. (CBD09-15)	UAIC 15298.01 (AC0915)	AC0915	AC0915	AC0915	AC0915
<i>Eremichthys acros</i>	Soldiers Meadow Spring, Lahontan Basin, Humboldt Co., Nevada, USA.	UAIC 13007.01 (EA13007)	EA13007	EA13007	EA13007	EA13007
	Black Rock Ranch, Desert Soldier Meadows, Lahontan Basin, Humboldt Co., Nevada, USA.	LV17537 (EA537)	EA537	EA537	EA537	EA537
<i>Gila atraria</i>	Sevier River, Piute Co., Utah, USA.	(SN86)	SN86	SN86	SN86	SN86
	Sevier River, Piute Co., Utah, USA.	(ATE17)	ATE17	ATE17	ATE17	ATE17
	Gandy Spring, Bonneville Basin, Tooele Co., Utah, USA.	(UTGA1)	UTGA1	UTGA1	UTGA1	UTGA1
<i>G. breviceauda</i>	Rio Candamena just downstream of Baseachi, Rio Mayo, Chihuahua, MX.	UAIC 15346.01 (GB0650)	GB0650	GB0650	GB0650	GB0650
	Rio Mayo drainage. Trib. W of Baseachic, Chihuahua, MX. (DAH2006-03-25-1)	(GP251)	GP251	GP251	GP251	GP251
<i>G. coerulea</i>	Upper Klamath Lake, Klamath Co., Oregon, USA.	OS 15083-1 (GC0831)	GC0831	GC0833	GC0833	GC0833
		OS 15082-3 (GO0833)				
	Upper Klamath Lake, Klamath Co., Oregon, USA.	BYU 239498 (GC498)	GC498	GC498	GC498	GC498
<i>G. conspersa</i>	Medina, Nazas River, MX.	(MNCN1444)	1444	1444	1444	1444
	Trib. Rio Aguanaval, Near Atonilto, Zacatecas, MX. (NUL02-134)	(G134)	G134	G134	G134	G134
<i>G. cypha</i>	Colorado River, Coconino Co., Arizona, USA.	(GC-2)	GC2	GC2	GC2	GC2
	Dolores River, Montrose Co., Colorado, USA. (Identified as <i>G. robusta</i>)	BYU 88870 (GR122012)	GR122012	GR122012	GR122012	GR122012
<i>G. diatenia</i>	Arroyo Atascosa at Hwy 15, South from Nogales. Rio de la Concepcion. Sonora, MX. (CBD0916)	UAIC 15299.02 (GD0916)	GD0916	GD0916	GD0916	GD0916
	Bridge Hwy 15. Rio de la Concepcion. Sonora, MX. (CBD09-15)	UAIC 15298.02 (GD0915)	GD0915	GD0915	GD0915	GD0915
<i>G. elegans</i>	Dexter National Fish Hatchery, New Mexico, USA.	(GE-01)	GE01	GE01	GE01	GE01
	Achili Hanyo, National Fish Hatchery, Arizona, USA.	(GE9061)	GE9061	GE9061	GE9061	GE9061
<i>G. eremica</i>	Rio Matape, just W San Jose de Pimas on Hwy 16. Sonora, MX. (CBD09-13)	UAIC 15296.01 (G09132)	G09132	G09132	G09132	G09132
	Arroyo San Miguel, Rio Sonora, Sonora, MX. (CBD0914)	UAIC 15297.03 (GE0914)	GE0914	GE0914	GE0914	GE0914
<i>G. intermedia</i>	Spring Creek tributary to Verde River, near Cottonwood, Arizona, USA.	(GSP9091)	GSP9091	GSP9091	GSP9091	GSP9091
<i>G. minacae</i>	Rio Bavispe at Tres Rios, Rio Yaqui, Sonora, MX. (DAN05-19)	UAIC 14970.02 (DAN519)	DAN519	DAN519	DAN519	DAN519
	Arroyo El Cocono at Hwy between Mesa Tres Rios and Largo, Rio Negro, Yaqui, Sonora, MX. (BRK07-97)	UAIC 14278.03 (GM07201)	GM07201	GM07201	GM07201	GM07201
<i>G. modesta</i>	Rio San Juan, 23 km Saltillo, Rio Grande, Coahuila, MX. (RLM05-61)	UAIC 15404.01 (MEX20)	MEX20	MEX20	MEX20	MEX20
	Los Chorrros, cerca Saltillo, Rio Santa Catalina, Coahuila, MX.	(MNCN 2462)	2462	2462	2462	2462
<i>G. nigra</i>	Marsh Creek, Gila Co., Arizona, USA.	(Marsa8)	Marsa8	Marsa8	Marsa8	Marsa8
	Fossil Creek above Irving, Yavapai Co, Arizona, USA.	(10fos125)	10fos125	10fos125	10fos125	10fos125
<i>G. nigrescens</i>	Unnamed tributary to Piedras Negras, Casas Grandes, Chihuahua, MX. (BRK07-89)	UAIC 14270.03 (GN0789)	GN0789	GN0789	GN0789	GN0789
	Rio Santa Maria, East Namiguipa on Hwy 15, Chihuahua, MX. (CBD09-04)	UAIC 15289.03 (GN0904)	GN0904	GN0904	GN0904	GN0904

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A. American western cyprinid genera (Continued)

Species	River	Voucher (Tissue)	Rag -1	Rhod	S - 7	Cyt b
<i>G. orcutti</i>	Sespe River, Santa Clara Dr., Ventura Co., California, USA. Rainbow Creek, San Diego Co., California, USA.	UAIC 11044.02 (GO-1) OS 15748-2 (GO748)	GO1 GO748	GO1 GO748	GO1 GO748	GO1 GO748
<i>G. pandora</i>	Rio Chama at US Hwy 84 near Arlequin, Rio Arriba Co., New Mexico, USA.	SLUM 662.01 (GP662) (PAN021)	GP662 PAN021	GP662 PAN021	GP662 PAN021	GP662 PAN021
<i>G. pulchra</i>	Rio Chama, Rio Grande Dr., Rio Arriba Co., New Mexico, USA. Arroyo de Recachi, Rio Conchos, Chihuahua, MX. (DLP05-5143)	(DLP5143) (G0715)	DLP5143 G0715	DLP5143 G0715	DLP5143 G0715	DLP5143 G0715
<i>G. purpurea</i>	Arroyo del Molino, Rio Conchos, Chihuahua, MX. (RLM07-03) Astin Spring Trans, Cosiche Co., Arizona, USA.	BYU 140722 (GP32251) BYU 140723 (GP32252) (GR9051)	GP32251 GP32252 GR9051	GP32251 GP32252 GR9051	GP32251 GP32252 GR9051	GP32251 GP32252 GR9051
<i>G. robusta</i>	Bubbling Ponds Fish Hatchery in Arizona, originally from Verde River near Cottonwood, Yavapai Co., Arizona, USA. Aravaipa Creek, Pinal Co., Arizona, USA.	(AVGR2) (CHGR8)	AVGR2 CHGR8	AVGR2 CHGR8	AVGR2 CHGR8	AVGR2 CHGR8
<i>G. seminuda</i>	Cherry Creek, Gila Co., Arizona, USA. Virgin River, Washington Co., Utah, USA. Virgin River at Littlefield, Mohave Co., Arizona, USA.	BYU 56251 (GS24701) (LIT4) (WFD4)	GS24701 LIT4 WFD4	GS24701 LIT4 WFD4	GS24701 LIT4 WFD4	GS24701 LIT4 WFD4
<i>Hesperoleucus symmetricus</i>	Washington Fields, Washington Co., Utah, USA. Kings River, 2 mi SW at Alta Wier, San Joaquin River, Fresno Co., California, USA. Alameda Creek, California, USA.	UAIC 11547.03 (HS11547) (Hes1, Hes2) (IO01, IO02)	HS11547 Hes1 Hes2 IO01 IO02	HS11547 Hes1 Hes2 IO01 IO02	HS11547 Hes1 Hes2 IO01 IO02	HS11547 Hes1 Hes2 IO01 IO02
<i>Iotichthys phlegathontis</i>	Private pond just west of Spanish Fork, Utah, USA.	(IP1)	IP1	IP1	IP1	IP1
<i>Lavinia exilicauda</i>	Gandy Spring, Bonneville Basin, Tooele Co., Utah, USA.	OS 015074 (SN88)	SN88	SN88	SN88	SN88
<i>Lepidomeda alvialis</i>	Putah Creek, Yolo Co., California, USA.	(lepab1)	lepab1	lepab1	lepab1	lepab1
<i>Lepidomeda vittata</i>	White River drainage, Nye Co., Nevada, USA. Rudd Creek Apache Co., Arizona, USA. Chevelon Creek, Coconino Co., Arizona, USA. East Clear Creek at Dines Tank, Coconino Co., Arizona, USA.	(SN89) (chevlv1) (DTiv1)	SN89 chevlv1 DTiv1	SN89 chevlv1 DTiv1	SN89 chevlv1 DTiv1	SN89 chevlv1 DTiv1
<i>Meda fulgida</i>	Aravaipa Creek, Gila River, Graham Co., Arizona, USA.	(SN85)	SN85	SN85	SN85	SN85
<i>Moapa coriacea</i>	Aravaipa Creek, Gila River, Graham Co., Arizona, USA. Muddy River, Clark Co., Nevada, USA.	(MF9011) UAIC 13165.01 (MC6, MC9)	MF9011 MC06 MC09	MF9011 MC06 MC09	MF9011 MC06 MC09	MF9011 MC06 MC09
<i>Mylocheilus caurinus</i>	North Thompson River at Little Fort, Fraser River, British Columbia, Canada. Snake River at mouth of Billingsley Creek at Lower Salmon Falls boat ramp, Goodwing Co., Idaho, USA. (BRK97-57)	UAIC 11553.01 (MC1993) UAIC 11633.01 (MC1997)	MC1993 MC1997	MC1993 MC1997	MC1993 MC1997	MC1993 MC1997
<i>Mylopharodon conocephalus</i>	Kern River, 16 km. above Kernville, Tulare Co., California, USA. Cottonwood Creek, Shasta-Tehama Cos., California, USA. (DAN07-43.01)	UAIC 11548.02 (CToL02622) (MC4301)	CToL2622 MC4301	CToL2622 MC4301	CToL2622 MC4301	CToL2622 MC4301
<i>Oregonichthys cramey</i>	Willamette, Shady Dell Pond, Lane Co., Oregon, USA. (SLUNUC050, Coll: Todd Pearson).	(OC050)	OC050	OC050	OC050	OC050
<i>Orthodon microlepidotus</i>	San Luis Reservoir, Merced Co., California, USA. (RLM96-59)	UAIC 11546.01 (OM9659) (PA9071)	CToL447 PA9071	CToL447 PA9071	CToL447 PA9071	CToL447 PA9071
<i>Plagopterus argenteus</i>	Dexter National Fish Hatchery Broodstock, originally from Virgin River, Mohave Co., Arizona, USA.					
<i>Pogonichthys macrolepidotus</i>	Montezuma sloop 1.2 km Montezuma, Colano Co., California, USA. (DAN07-16) Carquinez strait at Martinez, Contra Costa Co., California, USA. (DAN07-15)	(PM0716) (PM0715) (SN80)	PM0716 PM0715 SN80	PM0716 PM0715 SN80	PM0716 PM0715 SN80	PM0716 PM0715 SN80
<i>Ptychocheilus lucius</i>	No locality data. Dexter National Fish Hatchery Broodstock, New Mexico, USA.	(PL9041)	PL9041	PL9041	PL9041	PL9041

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A. American western cyprinid genera (Continued)

Species	River	Voucher (Tissue)	Rag -1	Rhod	S - 7	Cyt b
<i>Pychocheilus grandis</i>	Kings River 2 mi SW of Piedra at Alta Wier, San Joaquin River, Fresno Co., California, USA.	UAIC 11547.02 (PG11547)	PG11547	PG11547	PG11547	PG11547
	Kern River, 16 km. above Kernville, Tulare Co., California, USA. (RLM96-62)	UAIC 11548.01 (CTOL02631)	CTOL2631	CTOL2631	CTOL2631	CTOL2631
<i>Pychocheilus oregonensis</i>	Snake River at mouth of Billingsley Creek at Lower Salmon Falls boat ramp. Snake River. Gooding Co., Idaho, USA. (BRK97-57)	UAIC 11633.02 (PO9739)	PO9739	PO9739	PO9739	PO9739
	Silvies River downstream of dam, 4.7 mi N Burns (Malheur Lake), Harney Co., Oregon, USA. (BRK97-61)	UAIC 11650.02 (PO9761)	PO9761	PO9761	PO9761	PO9761
<i>Pychocheilus umpqua</i>	Umpqua R. Main stem, Douglas Co., Oregon, USA. (#43)	OS 17899 (PU43)	PU43	PU43	PU43	PU43
	Jackson Creek, South Umpqua R., Douglas Co., Oregon, USA (#154)	OS 17887 (PU154)	PU154	PU154	PU154	PU154
<i>Relictus solitarius</i>	Odgers Creek 15 miles W of Currie on Odgers Creek Ranch Road, Lahontan Dr., Elko Co., Nevada, USA.	UAIC 13005.01 (RS027)	RS027	RS027	RS027	RS027
	Odgers Creek, Elko Co., Nevada, USA.	OS 15745-1 (RS745)	RS745	RS745	RS745	RS745
	Odgers Creek, Elko Co., Nevada, USA.	(RS6881)	RS6881	RS6881	RS6881	RS6881
<i>Rhinichthys atratulus</i>	Russell Creek, Powell River, Claborn Co., Tennessee, USA.	UAIC 9850.01 (SN45)	SN45	SN45	SN45	SN45
<i>Rhinichthys cataractae</i>	Blue Creek at Hwy 26, 2 mi NW of Lewellen Garden Co., Nebraska, USA. (NJL03-236)	SLUM 491.02 (RC491)	RC491	RC491	RC491	RC491
<i>Rhinichthys osculus</i>	Cottonwood Creek at Co. Rd. 22, Lake Co., Oregon, USA. (BRK 97-73)	UAIC 11652.02 (RO11652)	RO11652	RO11652	RO11652	RO11652
<i>Richardsonius balteatus</i>	Portneuf River at United States Hwy 30, 2.2 mi NW Lava Hot Springs. Snake River, Bannock Co., Idaho, USA. (DAN97-31)	UAIC 11625.03 (RB9748)	RB9748	RB9748	RB9748	RB9748
	North Umpqua River at I-5 at Winchester, Douglas Co., Oregon, USA. (BRK97-69)	UAIC 11657.03 (RB9769)	RB9769	RB9769	RB9769	RB9769
<i>Richardsonius egregius</i>	Humboldt River, Elko Co., Nevada, USA. (DAN08-17)	(RE817)	RE817	RE817	RE817	RE817
<i>Siphateles alvordensis</i>	Trout Creek, Alford Basin, Harney Co., Oregon, USA. (PMH9317-1)	(SA317)	SA317	SA317	SA317	SA317
<i>Siphateles bicolor</i>	Upper Klamath Lake, Klamath Co., Oregon, USA. (DAN07-90.03)	(SB9003)	SB9003	SB9003	SB9003	SB9003
complex	Sprague River, Klamath Basin, Klamath Co., Oregon, USA.	(ORB1)	ORB1	ORB1	ORB1	ORB1
	Upper Klamath Lake, Oregon, USA. (Population 7459)	BYU 239544 (SIB1544)	SIB1544	SIB1544	SIB1544	SIB1544
	Humboldt River, Lahontan Basin, Persing Co., Nevada, USA.	OS 15637-3 (SO637)	SO637	SO637	SO637	SO637
	Pyramid Lake, Washoe Co., Nevada, USA.	(PYRB12)	PYRB12	PYRB12	PYRB12	PYRB12
	Warm Spring Ranch, Independence Basin, Elko Co., Nevada, USA.	OS 15622-1 (SI622)	SI622	SI622	SI622	SI622
	China Lake Naval Sta., Mohave Basin, San Bernardino Co., California, USA. (BB043)	(SBM43)	SMB43	SMB43	SMB43	SMB43
	Circle Ranch, Newark Basin, White Pine Co., Nevada, USA.	OS 15744-1 (SN744)	SN744	SN744	SN744	SN744
	Rock Creek, Catlow Basin, Harney Co., Oregon, USA.	OC 15635-7 (SE635)	SE635	SE635	SE635	SE635
<i>Siphateles boraxobius</i>	Borax Lake, Alvord Basin, Harney Co., Oregon, USA. (PMH9316-011)	(SB316)	SB316	SB316	SB316	SB316
<i>Snyderichthys copei</i>	Sevier River Dr., Garfield Co., Utah, USA.	(cop1UT1A)	cop1UT1A	cop1UT1A	cop1UT1A	cop1UT1A
	Spanish Fork River drainage, Utah Co., Utah, USA.	(SF1)	SF1	SF1	SF1	SF1
	Sulphur Creek, Bear River, Uinta Co., Wyoming, USA.	(SC178)	SC178	SC178	SC178	SC178
<i>Tiaroga cobitis</i>	Gila River, Colorado Dr., Grant Co., New Mexico, USA.	UAIC 13006.01 (SN59)	SN59	SN59	SN59	SN59
	Aravaipa Creek, Gila River, Colorado Dr., Graham Co., Arizona, USA.	(TC9021, TC9022)	TC9021	TC9021	TC9021	TC9021
			TC9022	TC9022	TC9022	TC9022
<i>Yurinia alta</i>	La Minizita Dam, Lerma Dr., Michoacan, MX.	(MNCN3809)	3809	3809	3809	3809
	Zacapu Lake, Lerma Dr., Michoacan, MX.	(MNCN3449)	3449	3449	3449	3449

B. American non-western cyprinids

<i>Chrosomus erythrogaster</i>	Spring River, Lawrence Co., Missouri, USA. (RLM83-19)	UAIC 11560.01	EF452834	EF452905765	GU134250	AY281055
<i>Clinostomus funduloides</i>	Shavers Creek, Randolph Co., West Virginia, USA. (BRK96-32)	UAIC 11403.02 (CFBRK32)	CFBRK32	CFBRK32	CFBRK32	CFBRK32
	Mill Creek, just NW of Old Ford (Catawba River) Santee River Dr., McDowell Co., North Carolina, USA. (RLM87-49)	UAIC 7920.02 (CF8749)	CF8749	CF8749	CF8749	CF8749
<i>Couesius plumbicus</i>	Mill Creek, 100 mi N of Fort Nelson, Mackenzie River, British Columbia, Canada.	UAIC 11366.01 (SN15)	SN15	SN15	SN15	SN15
<i>Erimonax monachus</i>	Buffalo River at Grinders Creek, Lewis Co., Tennessee, USA. (RLM93-13)	UAIC 10655.02 (SN52)	SN52	SN52	SN52	SN52
<i>Exoglossum laurae</i>	North Fork New River along Roy Kemp Road, Ashe Co., North Carolina, USA. (DAN98-72)	UAIC 12133.03 (EL12133)	EL12133	EL12133	EL12133	EL12133
<i>Exoglossum maxillingua</i>	Mettawee River, Lawrence River Dr., Washington Co., New York, USA. (AMS91-83)	UAIC 10527.02 (EM9183)	EM9183	EM9183	EM9183	EM9183
<i>Hybopsis winchelli</i>	Chappeela Creek at Hwy 40, 5 mi SE of Loranger, Tangipahoa Co., Louisiana, USA. (NJL03-353)	SLUM 673.02 (HW673)	HW673	HW673	HW673	HW673
<i>Margariscus margarita</i>	Plover River, Wisconsin River Dr., Marathon Co., Wisconsin, USA.	UAIC 10241.07 (CTOL0421)	CTOL0421	CTOL0421	CTOL0421	CTOL0421
<i>Notemigonus crysoleucas</i>	Mill Creek at County Road M., Portage Co., Wisconsin, USA. SN24, CTOL00435	UAIC 10527.02 (SN24)	SN24	SN24	SN24	SN24
<i>Platygobio gracilis</i>	Little White River, Melette Co., South Dakota, USA.	UAIC 11169.03 (SN56)	GU136347	SN56	GU134253	SN56
	Canadian River, Quay Co., New Mexico, USA.	UAIC 11559.01 (SN43)	GU136348	SN43	GU134252	EU811100

C. Related far-eastern cyprinids

<i>Tribolodon ezoe</i>	No locality data	(CTOL: 01745)	MIY18	MIY18	EU392223	MIY18
<i>Tribolodon hakonensis</i>	Tsukinuno River, tributary to Mogami River, Yamagata, Sea of Japan, Asia. Coll.: Harumi Sakai (8 oct 1997)	UAIC 11840.02 (TBH1997, CTOL: 00591)	CTOL0591	CTOL0591	TBH1997	CTOL0591